EXHIBIT B

abiotic or biotic stress; isolating MAPK5 protein from the plant; detecting for MAPK5 activity; and evaluating the increase or decrease in MAPK5 activity in the plant whereby the increase in MAPK5 activity indicates the plant is tolerant to stress. MAPK5 or its ortholog is isolated by immunoprecipitating the protein with a MAPK5 protein that specifically binds to MAPK5.

This invention also provides methods for enhancing tolerance to abiotic stress or increasing resistance to biotic stress in a plant. These methods include transforming a plant with MAPK5 nucleic acid sequence wherein the MAPK5 protein is expressed in the plant; treating a plant with an abiotic stress; isolating MAPK5 protein from the plant; detecting for MAPK5 activity; and evaluating the increase or decrease in MAPK5 activity in the transformed plant whereby the increase in MAPK5 activity indicates the increase in tolerance to abiotic stress in the transformed plant compared to the wild-type plant The decrease in MAPK5 activity indicates the increase resistance to biotic stress in the transformed plant compared to the wild-type plant.

The present invention also provides kits for screening plants for susceptibility to biotic stress or tolerance to abiotic stress. The kit includes an isolated nucleic acid probe that comprises a label and a nucleotide sequence that encodes a polypeptide consisting essentially of the amino sequence of MAPK5 or its complement and at least one reagent suitable for detecting the presence of a nucleic acid molecule encoding MAPK5 whereby the changes in polymorphic patterns of MAPK5 indicates the plant is susceptible to biotic stress. Another kit of the present invention provides for detecting a plant for tolerance to abiotic stress comprising an antibody that immunospecifically binds to a MAPK5 polypeptide wherein the antibody is labeled and at least one reagent suitable for detecting the presence of MAPK5 whereby the increase or decrease in MAPK5 activity indicates the plant is tolerant to abiotic stress.

5. DETAILED DESCRIPTION OF THE FIGURES

Figure 1 represents an amino acid sequence comparison of *OsMAPK5a* and *OsMAPK5ab* with MAPKs from other higher plants. (A) Alignment of deduced amino acid sequences of *OsMAPK5a* and *OsMAPK5b*, SEQ. ID. NOs:2 and 4, respectively, with two closely related MAPKs, TaWCK-1 and NtWIPK, SEQ. ID. NOs:9 and 10,

respectively. Conserved amino acid residues are listed. The 11 subdomains of the protein kinases are indicated above the sequences by Roman numbers. Threonine (T) and tyrosine (Y), two residues normally phosphorylated for activation of MAP kinases, are marked by *asterisks*. (B) The phylogenetic relationship of *OsMAPK5a* and *OsMAPK5b* with other plant MAPKs.

Figure 2 represents genomic organization, alternative splicing, recombinant proteins and autophosphorylation activity of *OsMAPK5*. (A) Southern blot analysis of the *OsMAPK5* gene. (B) RT-PCR analysis using a primer pair covering the differentiated regions of the *OsMAPK5a* and *OsMAPK5*b cDNAs. Lane 1 shows RT-PCR analysis of two days post infection blast fungus-induced mRNAs from the cultivar Drew. Lanes 2 and 3 represent PCR analysis of *OsMAPK5a* and *OsMAPK5b* cDNAs. (C) *In vitro* expression of *OsMAPK5a* and *OsMAPK5b*, and the specificity of the *OsMAPK5* antibody. One hundred nanograms of the total protein from *E. coli* (left lanes) or 10 ng (right lanes) of affinity-purified fusion protein of His-OsMAPK5a and His-OsMAPK5b were separated on 10% SDS-PAGE and detected with the anti-OsMAPK5 antibody. (D) *In vivo* autophosphorylation assay of affinity-purified fusion proteins, His-OsMAPK5a and His-*OsMAPK5b*.

Figure 3 represents activation of *OsMAPK5*, its protein and kinase activity by inoculation with the blast fungus. Assays were repeated three times using samples from independent experiments. (A) Northern blot analysis of *OsMAPK5* expression using the same gene-specific probe used in Southern blot analysis. Equal loading of total RNAs (20 μg per lane) was verified using rice 28S ribosomal RNA as a loading control. (B) Immunoblot analysis of OsMAPK5. (C) MBP in-gel kinase assay. Only the band corresponding to the activity of OsMAPK5a was shown since no activity was detected for OsMAPK5b. Avr and Vir denote avirulent and virulent isolates of the blast fungus, respectively.

Figure 4 represents induction of *OsMAPK5*, its protein and kinase activity by ABA and wounding. (A) Northern blot analysis of *OsMAPK5* expression in two-week-

sequence of SEQ. ID. NO: 4; or the complement of the nucleotide sequence of a polypeptide consisting of the amino acid sequence of SEQ. ID. NO: 4. It is understood that the complement of *OsMAPK5a* or *OsMAPK5b* or ortholog MAPK can be employed in this invention.

This invention includes an expression vector comprising the nucleotide sequence of SEQ.ID.NOs:1 or 2 or ortholog MAPK5 operatively associated with a regulatory nucleotide sequence containing transcriptional and translational regulatory information that controls expression of the nucleotide sequence in a host cell. The host expression vector system may include but not limited to microorganisms, insect, yeast or plants transformed with recombinant expression vectors.

The present invention provides a genetically host cell comprising the nucleotide sequences of SEQ. ID. NOs:1 or 3 or ortholog MAPK5. The genetically engineered host cell comprises an isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide consisting of the amino acid sequence of SEQ. ID. NO:2 or the complement of the nucleotide sequence of that encodes a polypeptide consisting of the amino acid sequence of SEQ. ID. NO:2. The genetically engineered host cell comprises an isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide consisting of the amino acid sequence of SEQ. ID. NO:4 or the complement of the nucleotide sequence of that encodes a polypeptide consisting of the amino acid sequence of SEQ. ID. NO:4. Moreover, the genetically engineered host cell of the present invention includes prokaryotic and eukaryotic cells.

In another embodiment, the genetically engineered host cell comprises the nucleotide sequences of SEQ. ID. NOs:1 or 3 or ortholog MAPK5 operatively associated with a regulatory sequence containing ~~transitional~~ transcriptional and translational regulatory information that controls expression of the nucleotide sequences in the host cell.

The present invention further provides an isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide consisting of the amino acid sequence of SEQ. ID. NO:2 or the complement of the nucleotide sequence of that encodes a polypeptide consisting of the amino acid sequence of SEQ. ID. NO:2 operatively associated with a regulatory sequence containing ~~transitional~~ transcriptional and

transformed plant whereby the decrease in MAPK5 activity indicates the increase resistance biotic stress in the transformed plant compared to the wild-type plant.

The present also provides kits for screening plants for susceptibility to biotic stress or tolerance to abiotic stress. One kit includes an isolated nucleic acid probe that comprises a label and (a) nucleotide sequence that encodes a polypeptide consisting essentially of the amino sequence of SEQ. ID. NO:2 or (b) the complement of (a). In another embodiment, the kit includes an isolated nucleic acid probe that comprises a label and (a) nucleotide sequence that encodes a polypeptide consisting of essentially the amino sequence of SEQ. ID. NO:4 or (b) the complement of (a).

The kit of the present invention provides for screening a plant for susceptibility to biotic stress comprising a nucleic acid probe and at least one reagent suitable for detecting the presence of a nucleic acid molecule encoding MAPK5 whereby the changes in polymorphic patterns of MAPK5 indicates the plant is susceptible to biotic stress. .

Another kit of the present invention provides for detecting a plant for tolerance to abiotic stress comprising an antibody that immunospecifically binds to a MAPK5 polypeptide wherein the antibody is labeled; and at least one reagent suitable for detecting the presence of MAPK5 whereby the increase or decrease in MAPK5 activity indicates the plant is tolerant to abiotic stress.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention.

7. MATERIALS AND METHODS

7.1 Isolation and Sequence Analysis of OsMAPK5

A full-length *OsMAPK5* cDNA was isolated using a 231? base pair *OsMAPK5* cDNA fragment (JB113) as a probe (Xiong et al., 2001). Approximately 106 plagues from a blast–induced cDNA library (Lee et al. 2001) were screened. The resulting positive clones carrying *OsMAPK5* cDNAs were excised *in vivo* from the lambda ZAP express vector with the aid of ExAssist helper phage (Startagene, La Jolla, CA). The full-length *OsMAPK5* cDNA clones were sequenced from both directions by a primer walking approach. Automated sequencing service was provided by the University of Arkansas for Medical Science. Sequence analysis was performed using Vector NT1